

## EXHIBIT B

United States Patent  
Cahoon , et al.

6,593,514  
July 15, 2003

Method for the production of calendic acid, a fatty acid containing delta-8,10,12 conjugated double bonds and related fatty acids having a modification at the delta-9 position

### Abstract

The preparation and use of nucleic acid fragments encoding plant fatty acid modifying enzymes associated with modification of the delta-9 position of fatty acids, in particular, formation of conjugated double bonds are disclosed. Chimeric genes incorporating such nucleic acid fragments and suitable regulatory sequences can be used to create transgenic plants having altered lipid profiles. The preparation and use of nucleic acid fragments encoding plant fatty acid modifying enzymes associated with formation of a trans delta-12 double bond also are disclosed. Chimeric genes incorporating such nucleic acid fragments and suitable regulatory sequences can be used to create transgenic plants having altered lipid profiles.

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**Field of Search:** 800/281,298 435/69.1,419 536/23.6

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*Parent Case Text*

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This application claims priority benefit of U.S. Provisional Application No. 60/149,050 filed Aug. 16, 1999, now abandoned.

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*Claims*

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What is claimed is:

1. A chimeric gene comprising an isolated *nucleic acid* fragment encoding a plant fatty acid modifying enzyme associated with conjugated double bond formation comprising a delta-9 position of fatty acids having an amino acid *identity* of at least 72.5% based on the Clustal method of alignment when compared to a polypeptide of SEQ ID NO:2 or 4 wherein said fragment or a functionally equivalent subfragment thereof or a complement thereof is operably linked to suitable regulatory sequences.
2. The chimeric gene of claim 1 wherein the *nucleic acid* fragment is isolated from

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Polypeptides having peroxidase activity and nucleic acids encoding same

**Abstract**

The present invention relates to isolated polypeptides having peroxidase activity and isolated nucleic acid sequences encoding the polypeptides. The invention also relates to nucleic acid constructs, vectors, and host cells comprising the nucleic acid sequences as well as methods for producing and using the polypeptides.

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001/68; C07H 021/04

**Field of Search:** 435/192,6,252.3,320.1 536/23.2,23.1

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***Parent Case Text***

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**CROSS-REFERENCE TO RELATED APPLICATION**

This application is a continuation-in-part of U.S. application Ser. No. 09/596,824 filed Jun. 19, 2000 now U.S. Pat. No. 6,372,464 issued Apr. 16, 2002, which application is fully incorporated herein by reference.

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***Claims***

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What is claimed is:

1. An isolated **nucleic acid** sequence encoding a polypeptide having peroxidase activity, selected from the group consisting of:
  - (a) a **nucleic acid** sequence encoding a polypeptide having an amino acid sequence which has at least 75% **identity** with amino acids 22 to 370 of SEQ ID NO:2 or amino acids 19 to 362 of SEQ ID NO:6, or at least 85% **identity** with amino acids 22 to 385 of SEQ ID NO:4;
  - (b) a **nucleic acid** sequence encoding a polypeptide having an amino acid sequence which has at least 75% homology with nucleotides 772 to 2302 of SEQ ID NO:1 or nucleotides 2848 to 4247 of SEQ ID NO:5, or at least 85% homology with nucleotides 2008 to 3462 of SEQ ID NO:3;
  - (c) a **nucleic acid** sequence which hybridizes under high stringency conditions with (i) nucleotides 772 to 2302 of SEQ ID NO:1, nucleotides 2008 to 3462 of SEQ ID NO:3, or nucleotides 2848 to 4247 of SEQ ID NO:5, (ii) the cDNA sequence contained in nucleotides 772 to 2302 of SEQ ID NO:1, nucleotides 2008 to 3462 of SEQ ID NO:3, or nucleotides 2848 to 4247 of SEQ ID NO:5, or (iii) a complementary strand of (i) or (ii); and
  - (d) a fragment of (a), (b), or (c), which encodes a polypeptide having peroxidase activity.
2. The **nucleic acid** sequence of claim 1, which encodes a polypeptide having an amino acid sequence which has at least 75% **identity** with amino acids 22 to 370 of SEQ ID NO:2 or amino acids 19 to 362 of SEQ ID NO:6.
3. The **nucleic acid** sequence of claim 2, which encodes a polypeptide having an amino acid sequence which has at least 80% **identity** with amino acids 22 to 370 of SEQ ID NO:2 or amino acids 19 to 362 of SEQ ID NO:6.
4. The **nucleic acid** sequence of claim 3, which encodes a polypeptide of having an amino acid sequence which has at least 85% **identity** with amino acids 22 to 370 of SEQ ID NO:2 or amino acids 19 to 362 of SEQ ID NO:6.
5. The **nucleic acid** sequence of claim 4, which encodes a polypeptide having an amino acid sequence which has at least 90% **identity** with amino acids 22 to 370 of SEQ ID NO:2 or amino acids 19 to 362 of SEQ ID NO:6.
6. The **nucleic acid** sequence of claim 5, which encodes a polypeptide having an amino acid sequence which has at least 95% **identity** with amino acids 22 to 370 of SEQ ID NO:2 or amino acids 19 to 362 of SEQ ID NO:6 .

7. The ***nucleic acid*** sequence of claim 1, which encodes a polypeptide comprising the amino acid sequence of SEQ ID NO:2, SEQ ID NO:4, or SEQ ID NO:6.
8. The ***nucleic acid*** sequence of claim 1, which encodes a polypeptide consisting of the amino acid sequence of SEQ ID NO:2, SEQ ID NO:4, or SEQ ID NO:6, or a fragment thereof having peroxidase activity.
9. The ***nucleic acid*** sequence of claim 1, which encodes a polypeptide consisting of the amino acid sequence of SEQ ID NO:2, SEQ ID NO:4, or SEQ ID NO:6.
10. The ***nucleic acid*** sequence of claim 1, which encodes a polypeptide which consists of amino acids 22 to 370 of SEQ ID NO:2, amino acids 22 to 365 of SEQ ID NO:4, or amino acids 19 to 362 of SEQ ID NO:6.
11. The ***nucleic acid*** sequence of claim 1, which has at least 75% homology with nucleotides 772 to 2302 of SEQ ID NO:1 or nucleotides 2848 to 4247 of SEQ ID NO:5.
12. The ***nucleic acid*** sequence of claim 11, which has at least 80% homology with nucleotides 772 to 2302 of SEQ ID NO:1 or nucleotides 2848 to 4247 of SEQ ID NO:5.
13. The ***nucleic acid*** sequence of claim 12, which has at least 85% homology with nucleotides 772 to 2302 of SEQ ID NO:1 or nucleotides 2848 to 4247 of SEQ ID NO:5.
14. The ***nucleic acid*** sequence of claim 13, which has at least 90% homology with nucleotides 772 to 2302 of SEQ ID NO:1 or nucleotides 2848 to 4247 of SEQ ID NO:5.
15. The ***nucleic acid*** sequence of claim 14, which has at least 95% homology with nucleotides 772 to 2302 of SEQ ID NO:1 or nucleotides 2848 to 4247 of SEQ ID NO:5.
16. The ***nucleic acid*** sequence of claim 1, which has the ***nucleic acid*** sequence of SEQ ID NO:1, SEQ ID NO:3, or SEQ ID NO:1.
17. The ***nucleic acid*** sequence of claim 1, which has the ***nucleic acid*** sequence of nucleotides 772 to 2302 of SEQ ID NO:1, nucleotides 2008 to 3462 of SEQ ID NO:3, or nucleotides 2848 to 4247 of SEQ ID NO:5.
18. The ***nucleic acid*** sequence of claim 1, which hybridizes under high stringency conditions with (i) nucleotides 772 to 2302 of SEQ ID NO:1, nucleotides 2008 to 3462 of SEQ ID NO:3, or nucleotides 2848 to 4247 of SEQ ID NO:5, (ii) me cDNA sequence contained in nucleotides 772 to 2302 of SEQ ID NO:1, nucleotides 2008 to 3462 of SEQ ID NO:3, or nucleotides 2845 to 4247 of SEQ ID NO:5, or (iii) a complementary strand of (i) or (ii).
19. The ***nucleic acid*** sequence of claim 1, which is contained in plasmid pBM37-7 which is contained in E. coli NRRL B-30280, plasmid pBM38-1 which is contained in E. coli NRRL B-30281, or plasmid pBM39-1 which is contained in E. coli NRRL B-30282.

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Plant polyphenol oxidase homologs

**Abstract**

This invention relates to an isolated nucleic acid fragment encoding a polyphenol oxidase enzyme. The invention also relates to the construction of a chimeric gene encoding all or a portion of the polyphenol oxidase enzyme, in sense or antisense orientation, wherein expression of the chimeric gene results in production of altered levels of the polyphenol oxidase enzyme in a transformed host cell.

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**021/04; C07H 021/04**  
Field of Search: **435/190,183,252.3,320.1,71.1 536/23.2,23.1**

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*Assistant Examiner:* Pak; Yong

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***Parent Case Text***

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This application claims the benefit of U.S. Provisional Application No. 60/119,590, filed Feb. 10, 1999.

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***Claims***

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What is claimed is:

1. An isolated polynucleotide comprising:

(a) a nucleotide sequence encoding a polypeptide having polyphenol oxidase B activity, wherein the polypeptide has an amino acid sequence of at least 80% *sequence identity*, based on the Clustal V method of alignment, when compared to SEQ ID NO:20, or

(b) a complement of the nucleotide sequence of (a), wherein the complement and the nucleotide sequence consist of the same number of nucleotides and are 100% complementary.

2. The polynucleotide of claim 1, wherein the amino acid sequence of the polypeptide has at least 85% *sequence identity*, based on the Clustal V method of alignment, when compared to SEQ ID NO:20.

3. The polynucleotide of claim 1, wherein the amino acid sequence of the polypeptide has at least 90% *sequence identity*, based on the Clustal V method of alignment, when compared to SEQ ID NO:20.

4. The polynucleotide of claim 1, wherein the amino acid sequence of the polypeptide has at least 95% *sequence identity*, based on the Clustal V method of alignment, when compared to SEQ ID NO:20.

5. The polynucleotide of claim 1, wherein the amino acid sequence of the polypeptide comprises SEQ ID NO:20.

6. The polynucleotide of claim 1 wherein the nucleotide sequence comprises SEQ ID NO:19.

7. A vector comprising the polynucleotide of claim 1.

8. A recombinant DNA construct comprising the polynucleotide of claim 1 operably linked to at least one regulatory sequence.

9. A method for transforming a cell, comprising transforming a cell with the polynucleotide of claim 1.

10. A cell comprising the recombinant DNA construct of claim 8.

11. A method for production of a polypeptide having polyphenol oxidase B activity comprising the steps of cultivating the cell of claim 10 under conditions that allow for the synthesis of the polypeptide and isolating the polypeptide from the cultivated cells, from the culture medium, or from both the cultivated cells and the culture medium.



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Human complement C3-degrading protein from Streptococcus pneumoniae

**Abstract**

The present invention relates to the identification and use of a family of human complement C3-degrading proteinases expressed by *S. pneumoniae*. The proteinase has a molecular weight of about 24 kD to about 34 kD as determined on a 10% SDS polyacrylamide gel. A preferred proteinase of this invention includes the amino acid sequence of SEQ ID NO:2.

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**424/244.1; 424/69.1; 424/320.1; 536/23.1; 536/23.7;**  
**530/324; 530/350; 530/380**  
Intern'l Class: **A61K 039/02**  
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**424/184.1,185.1,190.1,234.1,237.1,244.1,94.1 514/1**  
**536/23.1,23.2 435/69.1,320.1**

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**Foreign Patent Documents**

0 622 081	Nov., 1994	EP.
0 687 688	Dec., 1995	EP.

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***Government Interests***

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**STATEMENT OF GOVERNMENT SUPPORT**

The invention was made with the support of National Institutes of Health grant number R01-AI24162. The U.S. government may have certain rights to the invention.

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***Parent Case Text***

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This patent application claims benefit of Provisional application Ser. No. 60/044,316 filed Apr. 24, 1997.

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***Claims***

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What is claimed is:

1. An isolated and purified protein comprising at least an 80% sequence identity of SEQ ID NO:2 and that binds human complement protein C3.
2. The protein of claim 1 wherein the protein is isolated and purified from *S. pneumoniae*.
3. The protein of claim 1, wherein the protein is a recombinant protein.
4. The protein of claim 1 having a molecular weight as determined on a 10% polyacrylamide gel of between about 24 kDa to about 34 kDa.
5. The protein of claim 4, wherein the protein is isolated and purified from *S. pneumoniae*.
6. The protein of claim 4 wherein the protein is a recombinant protein.
7. The protein of claim 4 wherein the protein degrades human complement protein C3.
8. A peptide comprising at least 15 sequential amino acids from the protein of claim 1.
9. A composition comprising the peptide of claim 8.
10. The composition of claim 9, further comprising an adjuvant.

11. A composition comprising the protein of claim 1.
12. The composition of claim 11 further comprising an adjuvant.
13. An isolated and purified protein comprising SEQ ID NO:2.
14. A composition comprising the protein of claim 13.
15. The composition of claim 14 further comprising an adjuvant.
16. A peptide comprising at least 15 sequential amino acids set forth in SEQ ID NO:2, wherein said peptide binds human complement protein C3.
17. A composition comprising the peptide of claim 16.
18. The composition of claim 17 further comprising an adjuvant.
19. A protein comprising amino acids 1-50 of SEQ ID NO:2.
20. A composition comprising the protein of claim 19.
21. The composition of claim 20 further comprising an adjuvant.
22. An isolated and purified protein that binds human complement protein C3 and wherein nucleic acid encoding the protein hybridizes to SEQ ID NO:1 under hybridization conditions of 6.times.SSC, 5.times.Denhardt, 0.5% SDS, and 100 .mu.g/ml fragmented and denatured salmon sperm DNA hybridized overnight at 65.degree. C. and washed in 2.times.SSC, 0.1% SDS one time at room temperature for about 10 minutes followed by one time at, 65.degree. C. for about 15 minutes followed by at least one wash in 0.2.times.SSC, 0.1% SDS at room temperature for at least 3-5 minutes.
23. A composition comprising the protein of claim 22.
24. The composition of claim 23 further comprising an adjuvant.
25. An immunogenic composition comprising an isolated and purified polypeptide comprising SEQ ID NO:2.
26. The composition of claim 25 wherein the polypeptide is isolated and purified from *S. pneumoniae*.
27. The composition of claim 25 further comprising at least one other immune stimulating peptide, polypeptide or protein from *S. pneumoniae*.
28. An isolated nucleic acid fragment that hybridizes to SEQ ID NO:1 under hybridization

conditions of 6.times.SSC, 5.times.Denhardt, 0.5% SDS, and 100 .mu.g/ml fragmented and denatured salmon sperm DNA hybridized overnight at 65.degree. C. and washed in 2.times.SSC, 0.1% SDS one time at room temperature for about 10 minutes followed by one time at, 65.degree. C. for about 15 minutes followed by at least one wash in 0.2.times.SSC, 0.1% SDS at room temperature for at least 3-5 minutes, wherein said isolated nucleic acid fragment encodes a polypeptide that binds human complement protein C3.

29. The nucleic acid of claim 28 isolated from an *S. pneumoniae* genome.

30. The nucleic acid of claim 28 wherein the polypeptide degrades human complement C3.

31. The nucleic acid fragment of claim 28 wherein the nucleic acid fragment encodes a polypeptide that does not degrade human complement C3.

32. The nucleic acid of claim 28 in a nucleic acid vector.

33. The nucleic acid of claim 32 wherein the vector is an expression vector.

34. An isolated host cell comprising the nucleic acid vector of claim 32.

35. The cell of claim 34 wherein the cell is a bacterium or a eukaryotic cell.

36. An isolated host cell comprising the isolated nucleic acid of claim 28.

37. A method of expressing a polypeptide that binds to human complement protein C3, the method comprising culturing a recombinant host cell transformed with an isolated nucleic acid fragment of claim 28 under conditions suitable for expression of a polypeptide and recovering the polypeptide so expressed.

38. An isolated nucleic acid fragment comprising the nucleic acid sequence gctcccagtatgcgtactcgttaaggtagagggaagaaaaaactagctag (SEQ ID NO:9), wherein said isolated nucleic acid fragment encodes a polypeptide that binds human complement protein C3.

39. A method for producing an immune response to *S. pneumoniae* in an animal comprising the steps of:

administering a composition comprising a polypeptide comprising SEQ ID NO:2 to a mammal;  
and

obtaining an immune response to the polypeptide in said animal.

40. The method of claim 39 wherein the immune response is a B cell response.

41. The method of claim 39 wherein the immune response is a T cell response.

42. The method of claim 39 wherein the composition further comprises at least one other protein

from *S. pneumoniae*.

43. A bacteria comprising a nucleic acid comprising an insertional mutation, wherein said nucleic acid encodes a protein of claim 1.

44. The bacteria of claim 43 wherein the insertional mutation comprises an insertional duplication mutation.

45. An isolated and purified of about 24 kDa to about 34 kDa from *Streptococcus pneumoniae* that binds to human complement C3.

46. An isolated nucleic acid fragment comprising the nucleic acid sequence of SEQ ID NO:1.

47. The isolated nucleic acid fragment of claim 46 wherein the nucleic acid fragment encodes a protein that binds human complement C3.

48. An isolated RNA fragment transcribed from a double-stranded DNA sequence comprising SEQ ID NO:1.

49. An isolated nucleic acid fragment that encodes a polypeptide having at least an 80% *sequence identity* with SEQ ID NO:2 and binds human complement protein C3.

50. The isolated nucleic acid fragment of claim 49, said isolated nucleic acid fragment encoding a polypeptide comprising SEQ ID NO:2.

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Elongase genes and uses thereof

**Abstract**

The subject invention relates to the identification of several genes involved in the elongation of polyunsaturated acids (i.e., "elongases") and to uses thereof. At least two of these genes are also involved in the elongation of monounsaturated fatty acids. In particular, elongase is utilized in the conversion of gamma linolenic acid (GLA) to dihomogamma linolenic acid (DGLA) and in the conversion of AA to adrenic acid (ADA), or eicosapentaenoic acid (EPA) to .omega.3-docosapentaenoic acid (DPA). DGLA may be utilized in the production of polyunsaturated fatty acids, such as arachidonic acid (AA), docosahexaenoic acid (DHA), EPA, adrenic acid, .omega.6-docosapentaenoic acid or .omega.3-docosapentaenoic acid which may be added to pharmaceutical compositions, nutritional compositions, animal feeds, as well as other products such as cosmetics.

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C12N 005/06; C12N 005/16; C07H 021/04; 254.6; 252.31; 328; 254.23

**Field of Search:** 435/193,252.33,320.1,252.3,348,419,254.11,254.21,254.22,254.3,254.4,254.5  
536/23.2

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*Primary Examiner:* Prouty; Rebecca E.

*Assistant Examiner:* Swope; Sheridan

*Attorney, Agent or Firm:* Becker; Cheryl L.

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*Parent Case Text*

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The subject application is a Continuation-In-Part of U.S. patent application Ser. No. 09/624,670 filed on Jul. 24, 2000, which is a Continuation-In-Part of pending U.S. patent application Ser. No. 09/379,095 filed on Aug. 23, 1999, which is a Continuation-In-Part of U.S. patent application Ser. No. 09/145,828 filed on Sep. 2, 1998 now U.S. Pat. No. 6,403,349 issued Jun. 11, 2002, all of which are herein incorporated in their entirety by reference.

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*Claims*

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What is claimed is:

1. An isolated nucleic acid sequence comprising or complementary to a nucleic acid sequence encoding a polypeptide having elongase activity, wherein the amino acid sequence of said polypeptide has at least 80% amino acid *sequence identity* to SEQ ID NO:7.
2. The isolated nucleic acid sequence of claim 1 wherein said sequence comprises SEQ ID NO:7.
3. The isolated nucleic acid sequence of claims 1 or 2 wherein said sequence encodes a functionally active elongase which utilizes a polyunsaturated fatty acid as a substrate.
4. The isolated nucleic acid sequence of claim 1 wherein said sequence is derived from the genus *Thraustochytrium*.
5. The isolated nucleic acid sequence of claim 4 wherein said sequence is derived from *Thraustochytrium aureum*.
6. A method of producing an elongase enzyme comprising the steps of:
  - a) isolating a nucleotide sequence comprising SEQ ID NO:7 (FIG. 72);
  - b) constructing a vector comprising: i) said isolated nucleotide sequence operably linked to ii) a promoter;
  - c) introducing said vector into a host cell under time and conditions sufficient for expression of said elongase enzyme.
7. The method of claim 6 wherein said host cell is selected from the group consisting of a eukaryotic cell or a prokaryotic cell.
8. The method of claim 7 wherein said prokaryotic cell is selected from the group consisting of *E. coli*, *Cyanobacteria*, and *B. subtilis*.
9. The method of claim 7 wherein said eukaryotic cell is selected from the group consisting of a mammalian cell, an insect cell, a plant cell and a fungal cell.



10. The method of claim 9 wherein said fungal cell is selected from the group consisting of *Saccharomyces* spp., *Candida* spp., *Lipomyces starkey*, *Yarrowia* spp., *Kluyveromyces* spp., *Hansenula* spp., *Aspergillus* spp., *Penicillium* spp., *Neurospora* spp., *Trichoderma* spp. and *Pichia* spp.
11. The method of claim 10 wherein said fungal cell is a yeast cell selected from the group consisting of *Saccharomyces* spp., *Candida* spp., *Hansenula* spp. and *Pichia* spp.
12. The method of claim 11 wherein said yeast cell is *Saccharomyces cerevisiae*.
13. A vector comprising: a) a nucleotide sequence comprising SEQ ID NO:7 (FIG. 72) operably linked to b) a promoter.
14. A host cell comprising said vector of claim 13.
15. The host cell of claim 14 wherein said host cell is selected from the group consisting of a eukaryotic cell or a prokaryotic cell.
16. The host cell of claim 15 wherein said prokaryotic cell is selected from the group consisting of *E. coli*, *Cyanobacteria*, and *B. subtilis*.
17. The host cell of claim 15 wherein said eukaryotic cell is selected from the group consisting of a mammalian cell, an insect cell, a plant cell and a fungal cell.
18. The host cell of claim 17 wherein said fungal cell is selected from the group consisting of *Saccharomyces* spp., *Candida* spp., *Lipomyces starkey*, *Yarrowia* spp., *Kluyveromyces* spp., *Hansenula* spp., *Aspergillus* spp., *Penicillium* spp., *Neurospora* spp., *Trichoderma* spp. and *Pichia* spp.
19. The host cell of claim 18 wherein said fungal cell is a yeast cell selected from the group consisting of *Saccharomyces* spp., *Candida* spp., *Hansenula* spp. and *Pichia* spp.
20. The host cell of claim 19 wherein said yeast cell is *Saccharomyces cerevisiae*.
21. A plant cell comprising said vector of claim 13, wherein expression of said nucleotide sequence of said vector results in production of a polyunsaturated fatty acid by said plant cell.
22. The plant cell of claim 21 wherein said polyunsaturated fatty acid is selected from the group consisting of dihom- $\gamma$ -linolenic acid (DGLA), 20:4n-3, adrenic acid (ADA) and  $\omega$ -3-docosapentaenoic acid.

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Plant metabolism genes

Abstract

This invention relates to an isolated nucleic acid fragment encoding a GTP cyclohydrolase II/3,4-dihydroxy-2-butanone-4-phosphate synthase protein. The invention also relates to the construction of a chimeric gene encoding all or a substantial portion of the protein, in sense or antisense orientation, wherein expression of the chimeric gene results in production of altered levels of the protein in a transformed host cell.

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Inventors: **Allen; Stephen M.** (Wilmington, DE); **Kinney; Anthony J.** (Wilimington, DE); **Rafalski; J. Antoni** (Wilmington, DE); **Orozco, Jr.; Emil M.** (West Grove, PA); **Miao; Guo-Hua** (Johnston, IA); **Famodu; Omolayo O.** (Newark, DE); **Lee; Jian-Ming** (West Caldwell, NJ); **Lohman; Karin N.** (Newark, DE); **Rendina; Alan R.** (Wilmington, DE); **Sakai; Hajime** (Wilmington, DE); **Weng; Zude** (Des Plaines, IL); **Caimi; Perry G.** (Kennet Square, PA); **Fang; Yiwen** (Los Angeles, CA); **Shen; Jennie Bih-Jien** (Wilmington, DE); **Zoughi; Ilham L.** (Wilmington, DE); **Anderson; Shawn L.** (West Grove, PA); **Shi; Jinrui** (Johnston, IA); **Lu; Guihua** (Urbandale, IA); **Helentjaris; Timothy G.** (Ankeny, IA); **Li; Chun Ping** (Johnston, IA)

Assignee: **E.I. du Pont de Nemours and Company** (Wilmington, DE); **Pioneer Hi-Bred International** (Des Moines, IA)

Appl. No.: 614912

Filed: July 12, 2000

**Current U.S. Class:** 800/278; 435/6; 435/69.1; 435/183; 435/410; 435/419;  
435/252.3; 435/320.1; 530/350; 530/370; 536/23.2;  
536/23.6; 536/24.1; 800/295

**Intern'l Class:** A01H 003/00; C07H 021/04; C07K 014/415; C12N  
005/14; C12N 009/00

**Field of Search:** 435/6,69.1,183,410,419,252.3,320.1 530/350,370  
536/23.2,23.6,24.1 800/278,295

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*Primary Examiner:* Bui; Phuong T.

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### *Parent Case Text*

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This application claims the benefit of U.S. Provisional Applications No. 60/143,401 filed Jul. 12, 1999; No. 60/143,412, filed Jul. 12, 1999; No. 60/146,650, filed Jul. 30, 1999; No. 60/170,906 filed Dec. 15, 1999; No. 60/172,959 filed Dec. 21, 1999; No. 60/172,946 filed Dec. 21, 1999.

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### *Claims*

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What is claimed is:

1. An isolated polynucleotide comprising:

(a) a nucleotide sequence encoding a polypeptide having GTP cyclohydrolase II/3,4-dihydroxy-2-butanone-4-phosphate synthase activity, wherein the amino acid sequence of the polypeptide

and the amino acid sequence of SEQ ID NO:66 have at least 80% *sequence identity*, or

(b) the complement of the nucleotide sequence, wherein the complement and the nucleotide sequence contain the same number of nucleotides and are 100% complementary.

2. The polynucleotide of claim 1, wherein the amino acid sequence of the polypeptide and the amino acid sequence of SEQ ID NO:66 have at least 85% *sequence identity*.
3. The polynucleotide of claim 1, wherein the amino acid sequence of the polypeptide and the amino acid sequence of SEQ ID NO:66 have at least 90% *sequence identity*.
4. The polynucleotide of claim 1, wherein the amino acid sequence of the polypeptide and the amino acid sequence of SEQ ID NO:66 have at least 95% *sequence identity*.
5. The polynucleotide of claim 1, wherein the polypeptide comprises the amino acid sequence of SEQ ID NO:66.
6. The polynucleotide of claim 1, wherein the nucleotide sequence comprises the nucleotide sequence of SEQ ID NO:65.
7. A vector comprising the polynucleotide of claim 1.
8. A recombinant DNA construct comprising the polynucleotide of claim 1 operably linked to at least one regulatory sequence.
9. A method for transforming a cell comprising transforming a cell with the polynucleotide of claim 1.
10. A cell comprising the recombinant DNA construct of claim 8.
11. A method for producing a plant comprising transforming a plant cell with the polynucleotide of claim 1 and regenerating a plant from the transformed plant cell.
12. A plant comprising the recombinant DNA construct of claim 1.
13. A seed comprising the recombinant DNA construct of claim 1.

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Phosphatidylcholine biosynthetic enzymes

**Abstract**

This invention relates to an isolated nucleic acid fragment encoding phosphatidylethanolamine N-methyltransferase biosynthetic enzyme. The invention also relates to the construction of a chimeric gene encoding all or a portion of the phosphatidylethanolamine N-methyltransferase biosynthetic enzyme, in sense or antisense orientation, wherein expression of the chimeric gene results in production of altered levels of phosphatidylethanolamine N-methyltransferase biosynthetic enzyme in a transformed host cell.

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Inventors: **Famodu; Omolayo O.** (Newark, DE); **Kinney; Anthony J.** (Wilmington, DE); **Rafalski; J. Antoni** (Wilmington, DE)

Assignee: **E. I. du Pont de Nemours and Company** (Wilmington, DE)

Appl. No.: **668262**

Filed: **September 22, 2000**

**Current U.S. Class:** 800/281; 435/6; 435/69.1; 435/183; 435/410;  
435/419; 435/252.3; 435/320.1; 530/350; 530/370;  
536/23.2; 536/23.6; 536/24.1; 536/24.3; 536/24.33;  
800/278; 800/295

**Intern'l Class:** A01H 003/00; C07H 021/04; C07K 014/415; C12N  
005/14; C12N 009/00

**Field of Search:** 435/6,69.1,183,410,419,252.3,320.1 530/350,370  
536/23.2,23.6,24.1,24.3,24.33 800/278,295,281

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Bork. Genome Research, vol. 10, 2000, p. 398-400.

Primary Examiner: Bui; Phuong T.

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*Parent Case Text*

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This application claims the benefit of U.S. Provisional Application No. 60/155,626, filed Sep. 23, 1999.

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*Claims*

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What is claimed is:

1. An isolated ***polynucleotide*** comprising:

(a) a nucleotide sequence encoding a polypeptide having phosphatidylethanolamine N-methyltransferase activity, wherein the amino acid sequence of the polypeptide and the amino acid sequence of SEQ ID NO:20 have at least 80% sequence ***identity*** based on the Clustal alignment method, or

(b) the complement of the nucleotide sequence, wherein the complement and the nucleotide sequence contain the same number of nucleotides and are 100% complementary.

2. The ***polynucleotide*** of claim 1 wherein the amino acid sequence of the polypeptide and the amino acid sequence of SEQ ID NO:20 have at least 85% sequence ***identity*** based on the Clustal alignment method.

3. The ***polynucleotide*** of claim 1 wherein the amino acid sequence of the polypeptide and the amino acid sequence of SEQ ID NO:20 have at least 90% sequence ***identity*** based on the Clustal alignment method.

4. The ***polynucleotide*** of claim 1 wherein the amino acid sequence of the polypeptide and the amino acid sequence of SEQ ID NO:20 have at least 95% sequence ***identity*** based on the Clustal alignment method.

5. The ***polynucleotide*** of claim 1 wherein the polypeptide comprises the amino acid sequence of SEQ ID NO:20.

6. The ***polynucleotide*** of claim 1 wherein the nucleotide sequence comprises the nucleotide sequence of SEQ ID NO:19.

7. A vector comprising the *polynucleotide* of claim 1.
8. A recombinant DNA construct comprising the *polynucleotide* of claim 1 operably linked to a regulatory sequence.
9. A method for transforming a cell comprising transforming a cell with the *polynucleotide* of claim 1.
10. A cell comprising the recombinant DNA construct of claim 8.
11. A method for producing a plant comprising transforming a plant cell with the *polynucleotide* of claim 1 and regenerating a plant from the transformed plant cell.
12. A plant comprising the recombinant DNA construct of claim 8.
13. A seed comprising the recombinant DNA construct of claim 8.

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Nucleic acid molecules from plants encoding enzymes which participate in starch synthesis

**Abstract**

Nucleic acid molecules are described which encode enzymes which participate in starch synthesis in plants. These enzymes are a new isoform of starch synthase. There are furthermore described vectors for generating transgenic plant cells and plants which synthesize a modified starch. There are furthermore described methods for the generation of these transgenic plant cells and plants, and methods for producing modified starches.

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Inventors: **Frohberg; Claus** (Berlin, DE)

Assignee: **Aventis CropScience GmbH** (Frankfurt, DE)

Appl. No.: **638524**

Filed: **August 11, 2000**

**Foreign Application Priority Data**

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Aug 11, 1999[DE]	199 37 348
<b>Current U.S. Class:</b>	800/284; 800/278; 800/286; 800/320.1; 435/69.1; 435/101; 435/320.1; 435/419; 435/468; 536/23.6
<b>Intern'l Class:</b>	C12N 015/29; C12N 015/82; C12N 005/04; A01H 005/00; C12P 019/04
<b>Field of Search:</b>	536/23.6 435/69.1,468,320.1,419,101 800/278,284,320.1,286

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196 53 176	Jun., 1998	DE.
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WO 97/263362	Jul., 1997	WO.



WO 98/27212	Jun., 1998	WO.
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Database Accession No. C99345 published Oct. 16, 1998, also referred to as XP 002163786.  
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Cao, Heping et al, "Identification of the Soluble Starch Synthase Activities of Maize Endosperm", Plant Physiology, May 1999, vol. 120, pp. 205-215, No. 1.

*Primary Examiner:* Fox; David T.

*Attorney, Agent or Firm:* Frommer Lawrence & Haug LLP

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### Claims

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I claim:

1. An isolated **nucleic acid** molecule encoding a protein with the bioactivity of a starch synthase selected from the group consisting of

(a) **nucleic acid** molecules which encode a protein with the amino acid sequence indicated under SEQ ID No. 2;

(b) **nucleic acid** molecules which encompass the nucleotide sequence shown under SEQ ID No. 1 or a complementary sequence thereof;

(c) **nucleic acid** molecules which encompass the coding region of the nucleotide sequence of the cDNA present in plasmid IR 65/87 (deposit number DSM 12970) or a complementary sequence thereof;

(d) **nucleic acid** molecules whose nucleotide sequence deviates from the sequence of the **nucleic acid** molecules mentioned under (a), (b) or (c) owing to the degeneracy of the genetic code;

(e) **nucleic acid** molecules which have over 85% sequence **identity** with SEQ ID NO:1; and

(f) **nucleic acid** molecules which constitute allelic variants of the nucleic acid molecules

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Plant glucose-6-phosphate translocator**Abstract**

This invention relates to an isolated nucleic acid fragment encoding a glucose-6-phosphate/phosphate translocator. The invention also relates to the construction of a chimeric gene encoding all or a portion of the glucose-6-phosphate/phosphate translocator, in sense or antisense orientation, wherein expression of the chimeric gene results in production of altered levels of the glucose-6-phosphate/phosphate translocator in a transformed host cell.

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Inventors: **Allen; Stephen M.** (Wilmington, DE); **Rafalski; J. Antoni** (Wilmington, DE)

Assignee: **E. I. du Pont de Nemours and Company** (Wilmington, DE)

Appl. No.: **436521**

Filed: **November 9, 1999**

**Current U.S. Class:** 800/278; 435/6; 435/69.1; 435/71.1; 435/183;  
435/410; 435/419; 435/418; 435/252.3; 435/320.1;  
530/350; 530/370; 536/23.1; 536/23.2; 536/23.6;  
536/24.1; 536/24.3; 536/24.5

**Intern'l Class:** A01H 003/00; C07H 021/04; C07K 014/415; C12N  
005/14; C12N 009/00

**Field of Search:** 435/6,69.1,71.1,183,410,419,418,252.3,320.1  
530/370,350 536/23.1,23.2,23.6,24.1,24.3,24.5

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**Foreign Patent Documents**

WO 95/16913	Jun., 1995	WO.
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Bork. Genome Research vol. 10, p. 398-400, 2000.\*  
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Thorbjornsen et al. (1996) Plant J. 10:243-250.  
NCBI General Identifier No. 2997591.  
NCBI General Identifier No. 2997589.

*Primary Examiner:* Bui; Phuong T.

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*Parent Case Text*

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This application claims priority benefit to U.S. Provisional Application No. 60/107,910 filed Nov. 10, 1998, now abandoned.

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*Claims*

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What is claimed is:

1. An isolated *polynucleotide* comprising:

(a) a nucleotide sequence encoding a polypeptide having glucose-6-phosphate/phosphate translocator activity, wherein the amino acid sequence of the polypeptide and the amino acid sequence of SEQ ID NO:4 have at least 86% sequence *identity* based on the Clustal alignment method, or

(b) the complement of the nucleotide sequence, wherein the complement and the nucleotide sequence contain the same number of nucleotides and are 100% complementary.

2. The *polynucleotide* of claim 1, wherein the amino acid sequence of the polypeptide and the amino acid sequence of SEQ ID NO:4 have at least 90% sequence *identity* based on the Clustal alignment method.

3. The *polynucleotide* of claim 1, wherein the amino acid sequence of the polypeptide and the amino acid sequence of SEQ ID NO:4 have at least 95% sequence *identity* based on the Clustal alignment method.

4. The *polynucleotide* of claim 1, wherein the nucleotide sequence comprises the nucleotide sequence of SEQ ID NO:3.

5. The *polynucleotide* of claim 1, wherein the polypeptide comprises the amino acid sequence of SEQ ID NO:4.

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Method for activating only the vascular endothelial growth factor receptor-3 and uses thereof

**Abstract**

A method for activating only the vascular endothelial growth factor receptor-3 has been created. The method comprises administration of a composition comprising a polypeptide to an animal wherein the composition has the ability to stimulate one or more lymphatic vessel endothelial cells to proliferate, differentiate, migrate, or survive. Methods are also provided to selectively activate a VEGF receptor-3, to screen for neoplastic disease characterized by an increase in lymph vessel endothelial cells, and to identify lymph vessel endothelial cells.

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Inventors: **Achen; Marc** (Parkville, AU); **Stacker; Steven** (Parkville, AU)

Assignee: **Ludwig Institute for Cancer Research** (New York, NY)

Appl. No.: **847524**

Filed: **May 3, 2001**

**Current U.S. Class:**

**424/85.1; 530/351**

**Intern'l Class:**

**A61K 038/19**

**Field of Search:**

**424/85.1 530/351 435/7.1,325**

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**References Cited [Referenced By]**

**Other References**

Achen et al. VEGF-D is a ligand for the VEGF receptor 2 (Flk1) and VEGF receptor 3 (Flt4). 1998. Proc. Natl. Acad. Sci. USA, 95:548-553.

*Primary Examiner:* Spector; Lorraine

*Assistant Examiner:* Jiang; Dong

*Attorney, Agent or Firm:* Crowell & Moring LLP

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***Claims***

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What is claimed is:

1. A method for stimulating proliferation and/or maintaining of only lymph vessel endothelial cells, in a mammal in need of such treatment, comprising:

administering to said cells an effective amount of a composition comprising a polypeptide having at least a 90% *sequence identity* with the amino acid sequence of SEQ ID NO:2 or SEQ ID NO:4 or SEQ ID NO:6, or a fragment thereof which has the ability to only stimulate lymphatic vessel endothelial cells to proliferate, differentiate, migrate or survive.

2. The method of claim 1, wherein the polypeptide has at least a 95% *sequence identity* with the amino acid sequence of SEQ ID NO:2 or SEQ ID NO:4 or SEQ ID NO:6, or a fragment thereof.

3. The method of claim 2, wherein the polypeptide comprises the amino acid sequence of SEQ ID NO:2 or SEQ ID NO:4 or SEQ ID NO:6, or a fragment thereof.

4. The method of claim 1, wherein the polypeptide comprises the amino acid sequence of SEQ ID NO:2 or SEQ ID NO:4 or SEQ ID NO:6.

5. A method for activating only a VEGF receptor-3, comprising:

administering to a cell bearing said receptor an effective amount of a composition comprising a polypeptide having at least 90% *sequence identity* with the amino acid sequence of SEQ ID NO:2 or SEQ ID NO:4 or SEQ ID NO:6, or a fragment thereof which has the ability only to activate a VEGF receptor 3.

6. The method of claim 5, wherein the polypeptide has a 95% sequence identity with the amino acid sequence of SEQ ID NO:2 or SEQ ID NO:4 or SEQ ID NO:6, or a fragment thereof.

7. The method of claim 6, wherein the polypeptide comprises the amino acid sequence of SEQ ID NO:2 or SEQ ID NO:4 or SEQ ID NO:6, or a fragment thereof.

8. The method of claim 7, wherein the polypeptide comprises the amino acid sequence of SEQ ID NO:2 or SEQ ID NO:4 or SEQ ID NO:6.

9. The method of claim 5, wherein the method is carried out in vivo.

10. The method of claim 5, wherein the method is carried out in vitro.